REVIEWED
By Celena Shrum at 9:30 am, Nov 21, 2024

11/19/2024

Worklist: 6976

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION	
C2024-1731	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ	
C2024-2232	1	BCK	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ	

Panel 2 - AM# 28: Multi-Drug Quantitation by LC-MS/MS

Extraction Date: 11/19/24
Plate lot 240924
Mobile phase A: 5mM Amm Form + 0.01% FA
Blank Blood Lot: 24C52044
Column: Agilent 120 EC-C18 (2.1x 100-2.7um)

Analyst: Anne Nord
Plate Retest Date: 3/24/2025
Mobile phase B: 0.01% Formic Acid in MeOH
Blank Urine Lot: Blood only
LCMS-QQQ ID: 69679

Pre-Analytic:

☒ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
☒ 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
☒ 2. Using a calibrated pipette, pipette **250µL blood and urine** (if applicable) into wells of analytical (standards) plate. **Pipette ID: 390993** Urine Hydrolysis: add 250µL urine, 40µL BG Turbo, and 100µL Instant Buffer I. Place on plate shaker for 5 minutes
☒ 3. Pipette **250µL 0.5 M ammonium hydroxide** in wells of analytical plate.
☒ 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 5. Transfer **200-450µL of blood+base and urine+base (if applicable)** mixture to corresponding wells of SLE+ plate. Amount transferred: *250*
☒ 6. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). ***(Load at 85-100 PSI- Selector to the right).***
☒ 7. Wait 5 minutes.
☒ 8. Add **900uL ethyl acetate.**
☒ 9. Wait 5 minutes.
☒ 10. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 11. Add **900uL ethyl acetate.**
☒ 12. Wait 5 minutes.
☒ 13. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 14. Remove plate containing eluate. Place on SPE Dry 75401 and evaporate to dryness at approx. 35°C. ☐ If run contains urine or at the analyst's discretion, add 50µL 1% HCl in MeOH to wells and place plate cover on plate before drying (optional).
☒ 15. Reconstitute in **100µL 20% LC MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

☒ 1. Create batch and process data.
☒ 2. Make necessary changes to integration limits
☒ 3. Integration linear and R^2 values ≥0.98 for each analyte.
☒ 4. For unknown samples and controls: response ratio within 20% of average of controls and standards, RT within +/- 5% (tramadol RT +/-2%), S/N for primary transition >10 and secondary transitions >5.
☒ 5. Did all QCs pass for each analyte? Yes, see comments Add Control data to QC tracking spreadsheet.
☒ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS:
Compounds evaluated:

Methocarbamol
Clomipramine
Topiramate Curve limitation 5-500 cal 8 dropped due to accuracy

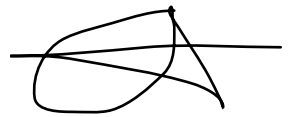
	1 mixing plate 5 SLE and Injection	2 mixing plate 6 SLE and Injection
A	IS + Cal. 1	IS + QC_1
B	IS + Cal. 2	IS + QC_2
C	IS + Cal. 3	IS + QC 3
D	IS + Cal. 4	IS + QC_4
E	IS + Cal. 5	
F	IS + Cal. 6	negative blood
G	IS + Cal. 7	1731-1
H	IS + Cal. 8	2232-1

11/21/24

See next page with correct positioning

plate position 2

c2024-____-_



	1 mixing plate 5 SLE and Injection	2 mixing plate 6 SLE and Injection
A	IS + Cal. 1	IS + QC_1
B	IS + Cal. 2	IS + QC_2
C	IS + Cal. 3	IS + QC 3
D	IS + Cal. 4	IS + QC_4
E	IS + Cal. 5	negative blood
F	IS + Cal. 6	1731-1
G	IS + Cal. 7	2232-1
H	IS + Cal. 8	

plate position 2

c2024-____-_

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 qc10 mdq.d
Type	QC	**Sample**	am 28 p2 qc10 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-A6	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 6:26:21 PM		
Sample Info.			

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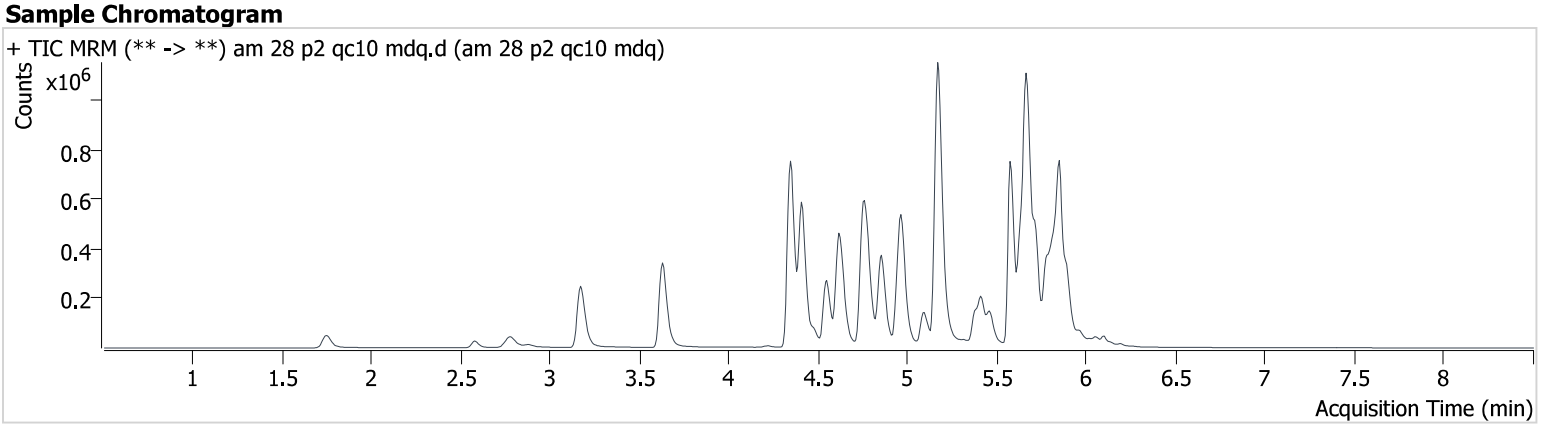
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	122804	913.9	90.29	935.2	620069	9.562	ng/ml
methocarbamol	4.380	12274	1287.1	75.10	277.1	756314	10.930	ng/ml
Topiramate	4.948	743	85.0	43.34	38.2	5882	11.131	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 qc 100 mdq.d
Type	Sample	**Sample**	am 28 p2 qc 100 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-B6	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 7:30:56 PM		
Sample Info.			

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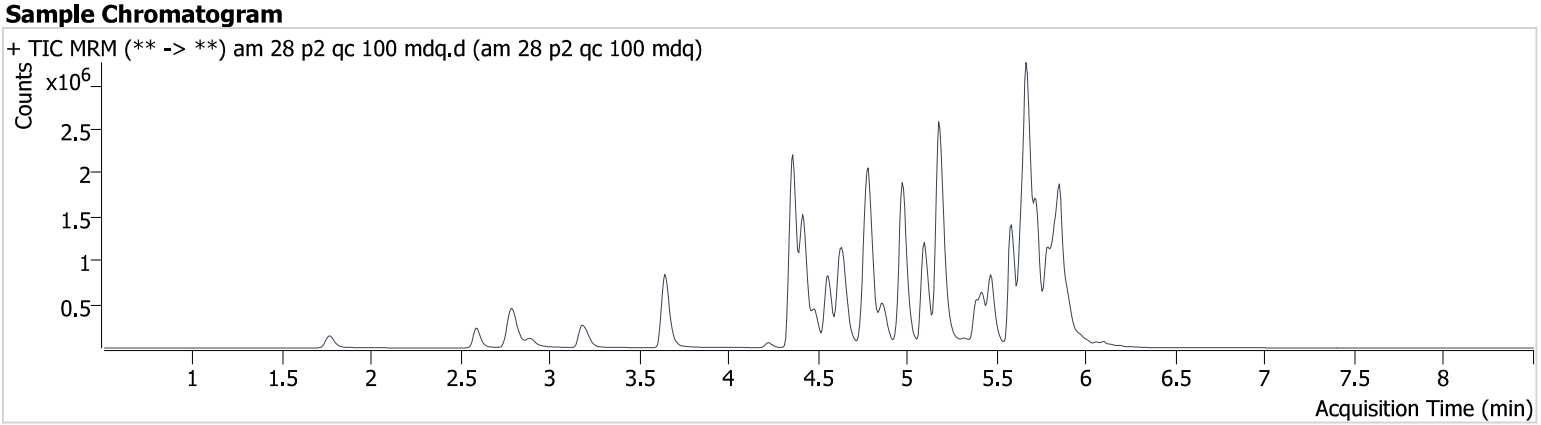
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	1028676	48355.7	90.64	13552.0	501997	96.300	ng/ml
methocarbamol	4.387	89613	2538.3	77.79	1368.1	653159	95.600	ng/ml
Topiramate	4.948	7243	268.0	43.65	189.8	5826	106.105	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 qc-250 mdq.d
Type	QC	**Sample**	am 28 p2 qc-250 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-C6	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 6:35:35 PM		
Sample Info.			

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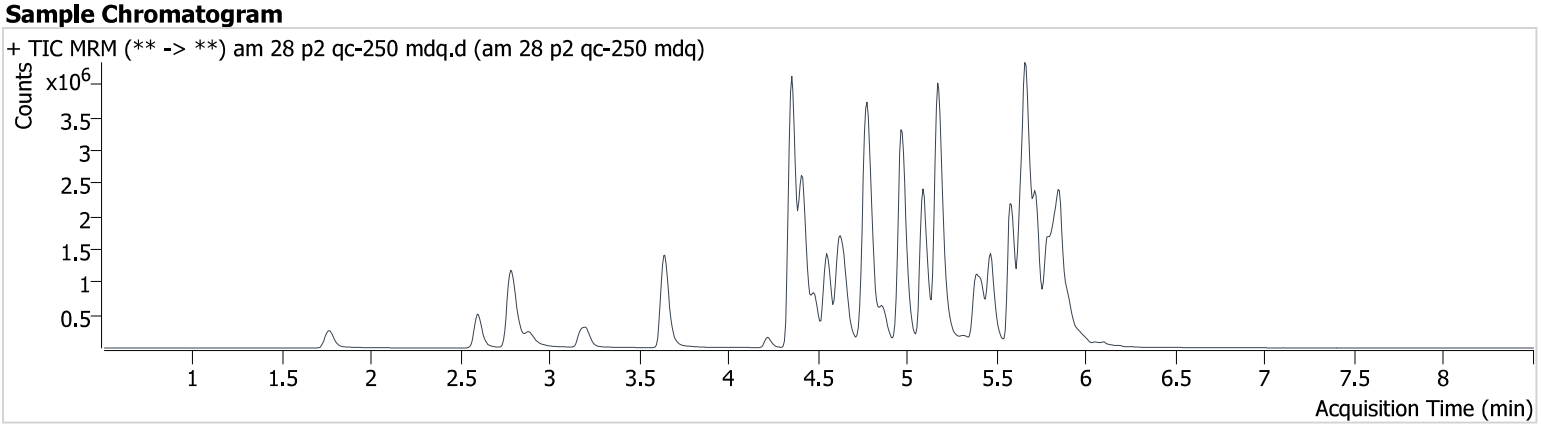
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	1519815	44301.1	87.80	52661.4	269369	264.655	ng/ml
methocarbamol	4.380	169749	55141.7	77.00	155200.4	385895	307.456	ng/ml
Topiramate	4.941	16814	1056.8	44.41	232.8	5957	240.421	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 qc-1000 mdq.d
Type	QC	**Sample**	am 28 p2 qc-1000 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-D6	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 6:44:49 PM		
Sample Info.			

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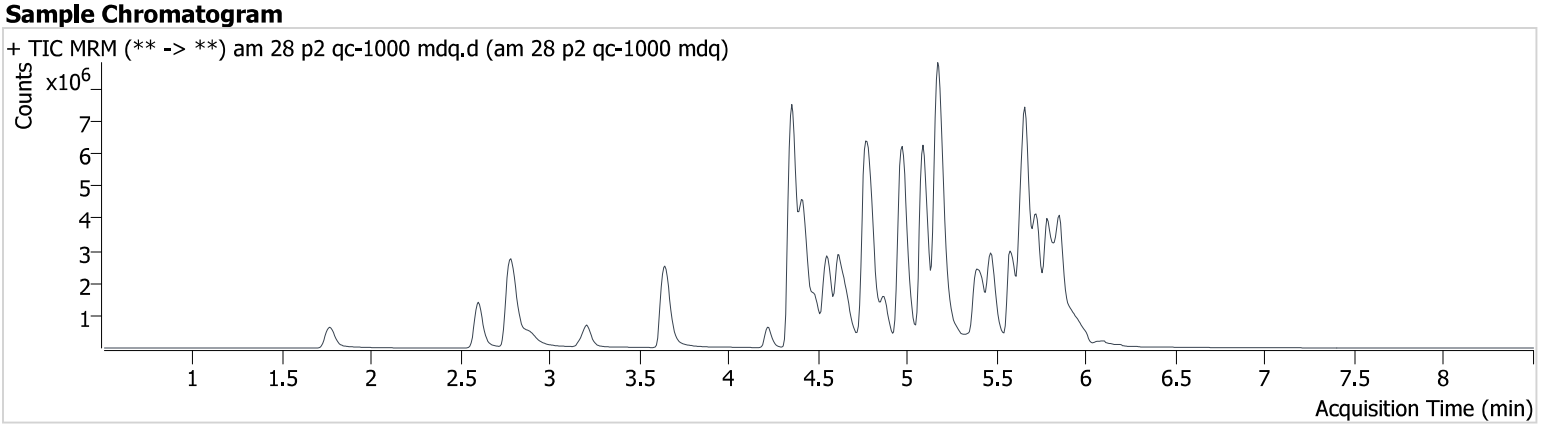
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	3036312	112478.8	88.42	97277.4	138853	1024.906	ng/ml
methocarbamol	4.380	242010	260505.1	79.59	317213.8	190419	889.129	ng/ml
Topiramate	4.935	40744	28711.8	43.61	4019.3	4767	727.203	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 negative blood.d
Type	Sample	**Sample**	am 28 p2 negative blood
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-E6	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 7:03:14 PM		
Sample Info.			

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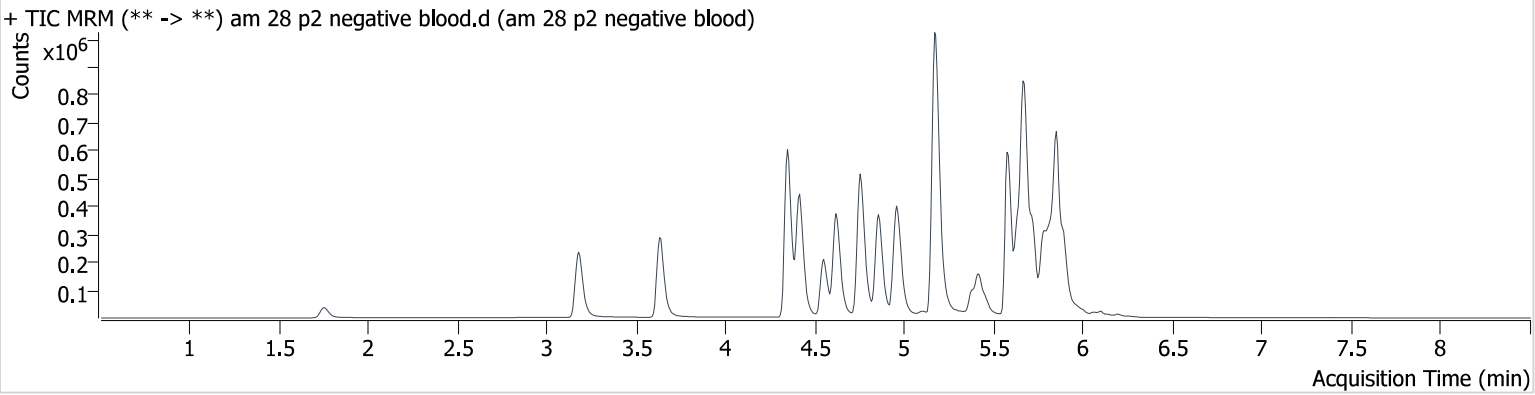
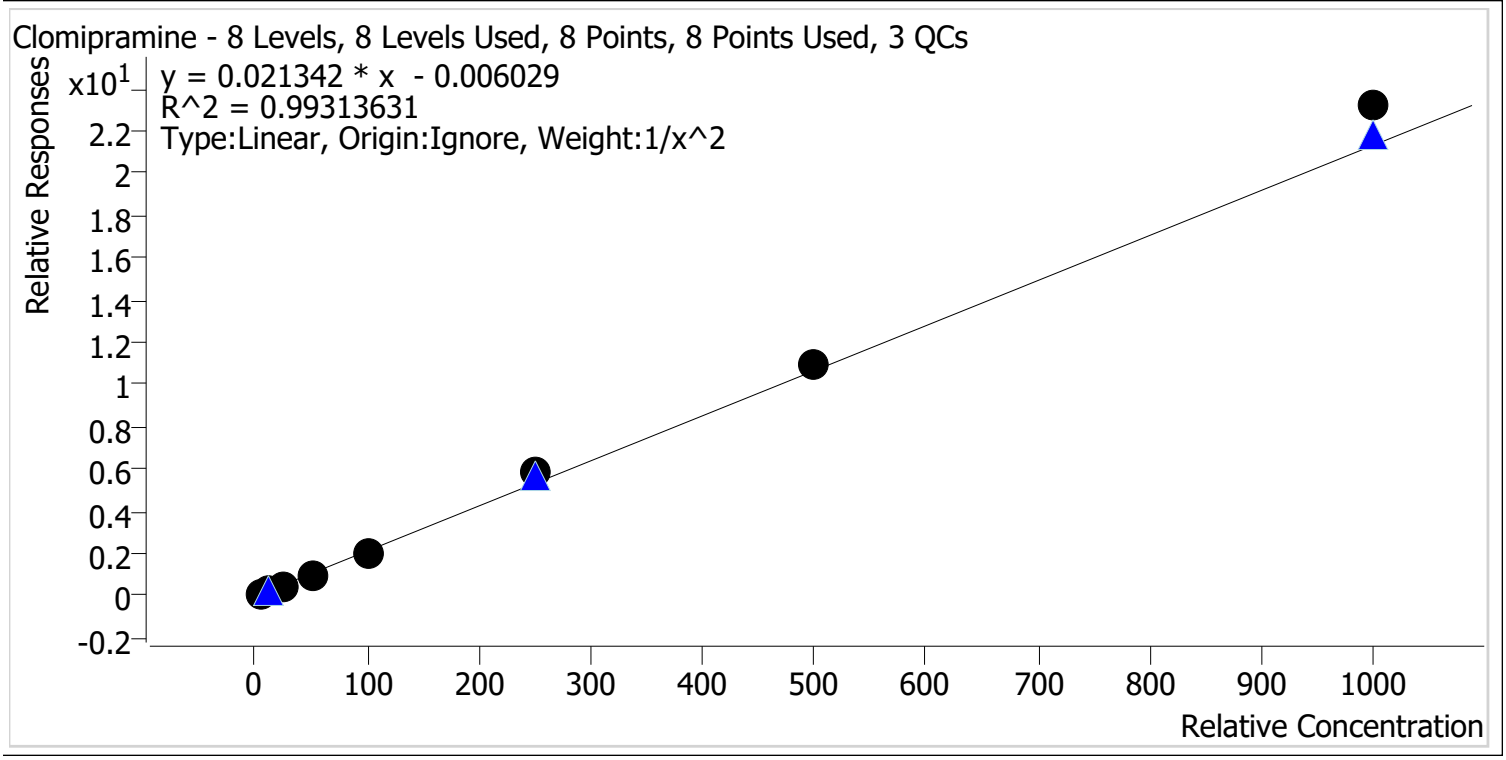
Sample Chromatogram



Compound Calibration Report

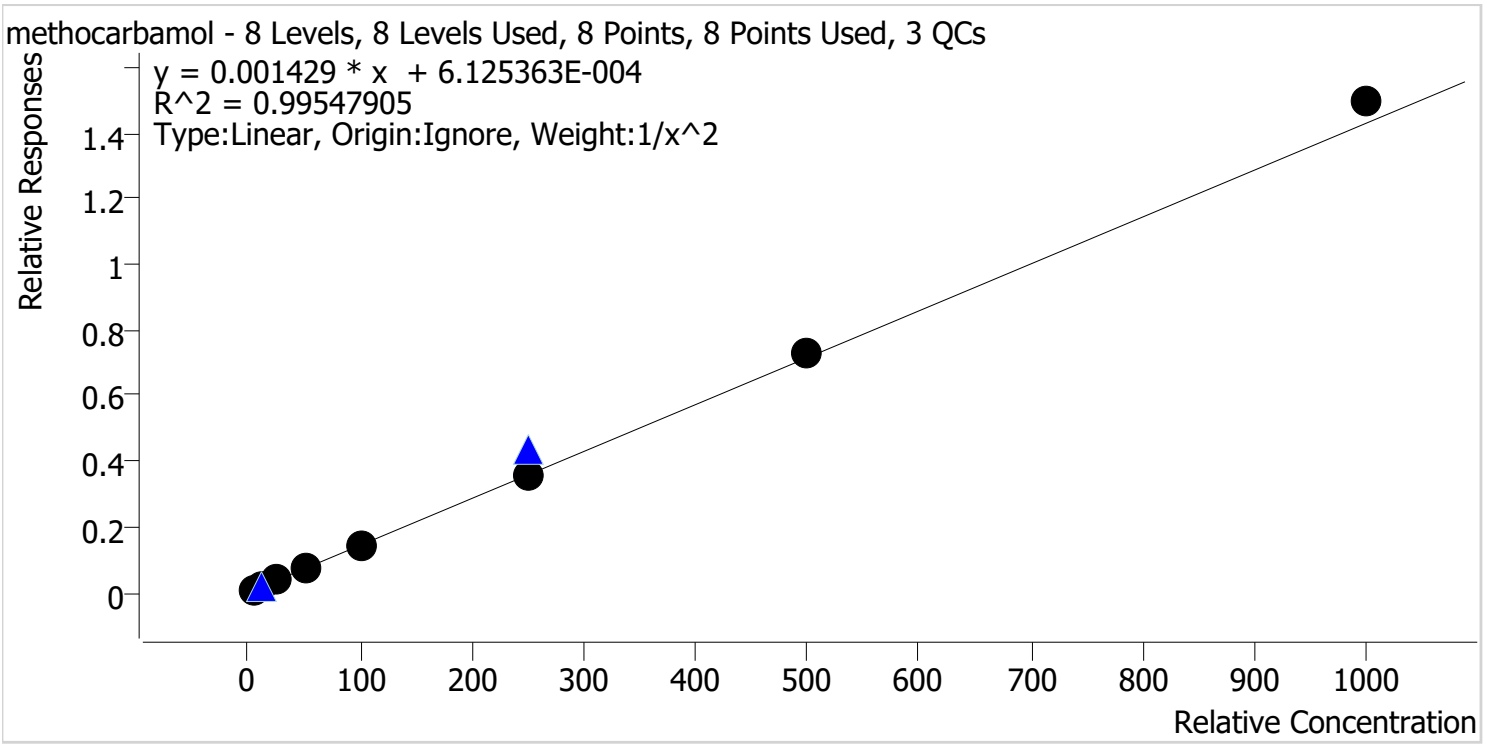
Batch results	D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Last Cal. Update	11/20/2024 4:33 PM
Analyst Name	ISP\datastor
Analyte	Clomipramine
Internal Standard	Clomipramine-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
am 28 p2 cal 1 mdq	1	✔	5.0	5.3	105.5
am 28 p2 cal 2 mdq	2	✔	10.0	9.3	93.2
am 28 p2 cal 3 mdq	3	✔	25.0	23.2	92.7
am 28 p2 cal 4 mdq	4	✔	50.0	46.8	93.5
am 28 p2 cal 5 mdq	5	✔	100.0	94.4	94.4
am 28 p2 cal 6 mdq	6	✔	250.0	271.3	108.5
am 28 p2 cal 7 mdq	7	✔	500.0	516.5	103.3
am 28 p2 cal 8 mdq	8	✔	1000.0	1088.2	108.8

Compound Calibration Report

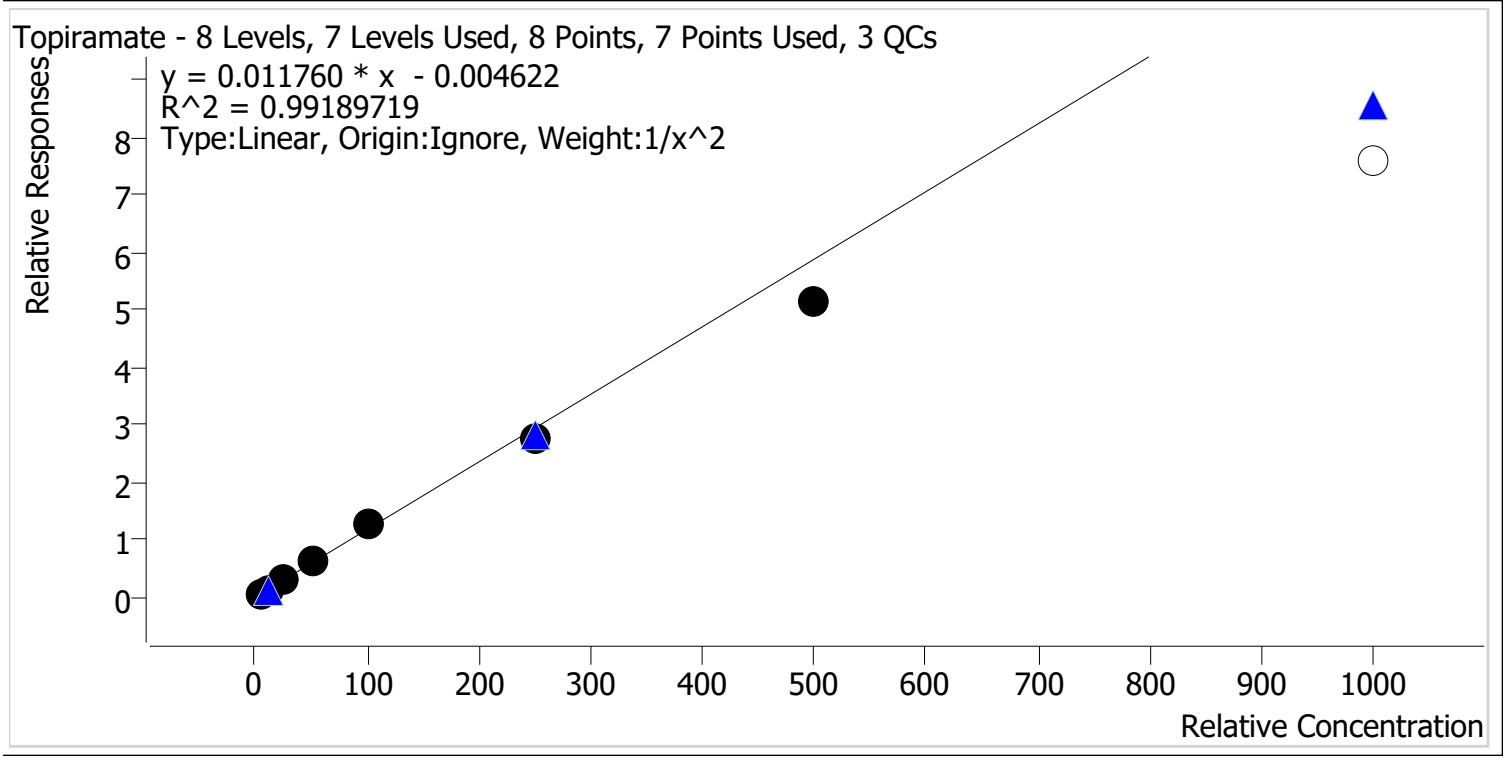
Batch results	D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Last Cal. Update	11/20/2024 4:33 PM
Analyst Name	ISP\datastor
Analyte	methocarbamol
Internal Standard	Imipramine-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
am 28 p2 cal 1 mdq	1	✔	5.0	5.3	106.1
am 28 p2 cal 2 mdq	2	✔	10.0	8.7	87.1
am 28 p2 cal 3 mdq	3	✔	25.0	25.1	100.5
am 28 p2 cal 4 mdq	4	✔	50.0	51.4	102.9
am 28 p2 cal 5 mdq	5	✔	100.0	98.7	98.7
am 28 p2 cal 6 mdq	6	✔	250.0	246.8	98.7
am 28 p2 cal 7 mdq	7	✔	500.0	506.7	101.3
am 28 p2 cal 8 mdq	8	✔	1000.0	1046.2	104.6

Compound Calibration Report

Batch results	D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Last Cal. Update	11/20/2024 4:33 PM
Analyst Name	ISP\datastor
Analyte	Topiramate
Internal Standard	Topiramate-d12



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
am 28 p2 cal 1 mdq	1	✔	5.0	4.9	97.8
am 28 p2 cal 2 mdq	2	✔	10.0	9.9	99.5
am 28 p2 cal 3 mdq	3	✔	25.0	27.6	110.4
am 28 p2 cal 4 mdq	4	✔	50.0	51.4	102.8
am 28 p2 cal 5 mdq	5	✔	100.0	107.2	107.2
am 28 p2 cal 6 mdq	6	✔	250.0	236.5	94.6
am 28 p2 cal 7 mdq	7	✔	500.0	439.0	87.8
am 28 p2 cal 8 mdq	8	✗	1000.0	648.1	64.8

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 1 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 1 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-A5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:03:18 PM		
Sample Info.			

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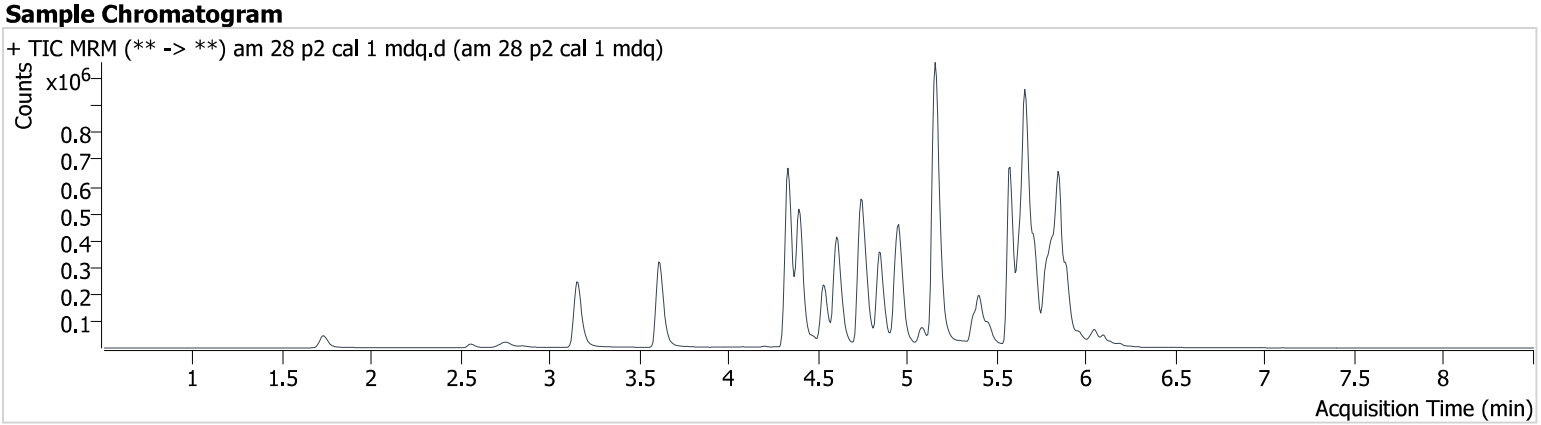
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.842	65657	1563.5	89.59	2233.2	615968	5.277	ng/ml
methocarbamol	4.367	6196	145.5	77.27	111.3	756179	5.306	ng/ml
Topiramate	4.935	308	8.0	53.77	13.9	5827	4.889	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 2 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 2 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-B5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:12:32 PM		
Sample Info.			

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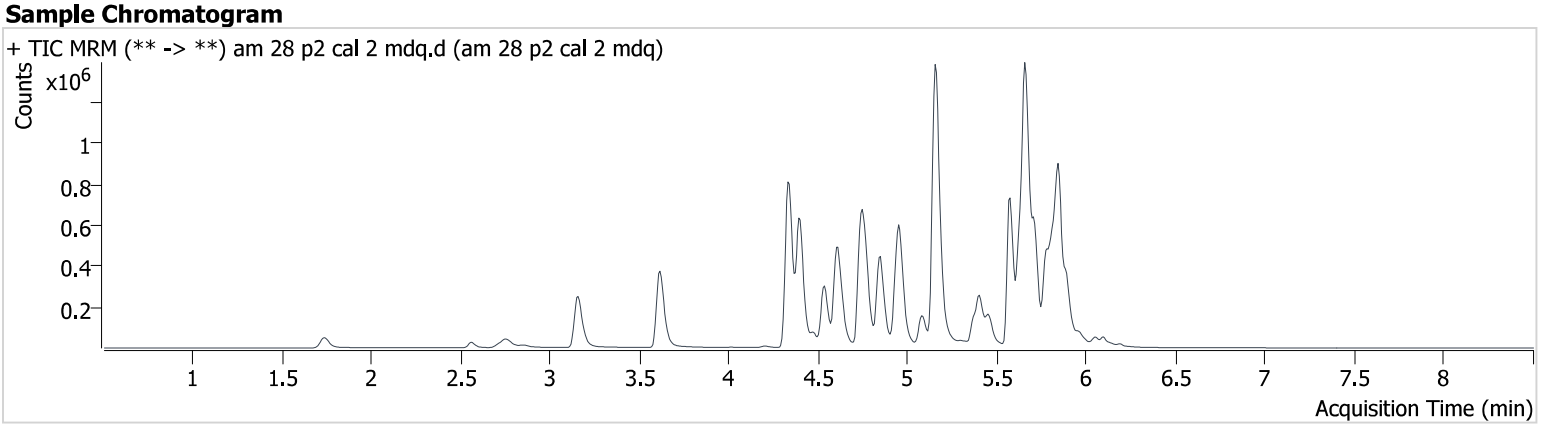
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.842	163275	94882.1	92.89	2236.5	846578	9.320	ng/ml
methocarbamol	4.367	12474	311.3	73.48	113.7	955655	8.707	ng/ml
Topiramate	4.935	666	51.9	49.26	26.1	5928	9.949	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 3 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 3 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-C5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:21:46 PM		
Sample Info.			

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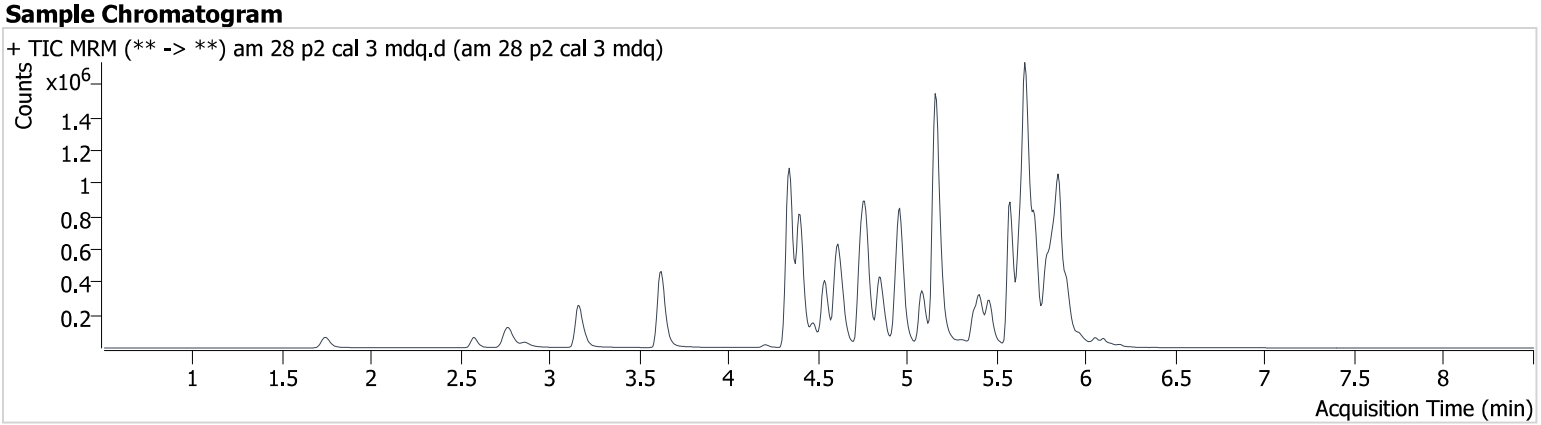
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.842	336172	239302.9	89.63	2092.7	688097	23.175	ng/ml
methocarbamol	4.367	29384	1007.6	75.60	4689.4	804457	25.137	ng/ml
Topiramate	4.935	1982	237.2	50.09	449.2	6196	27.594	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 4 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 4 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-D5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:30:59 PM		
Sample Info.			

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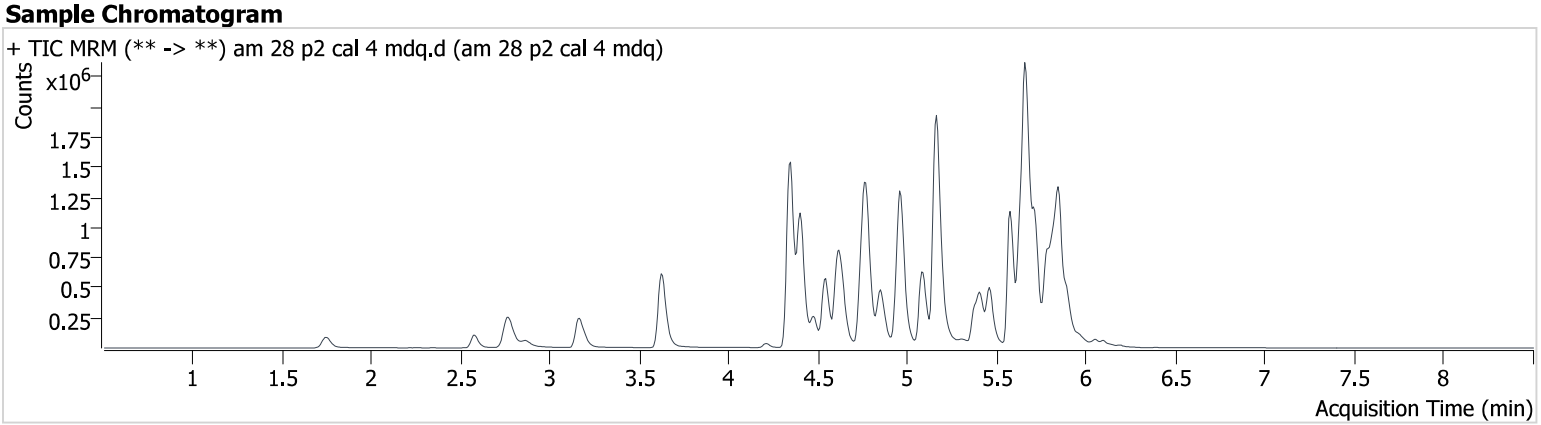
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.842	575487	12781.1	89.85	9892.4	580022	46.773	ng/ml
methocarbamol	4.373	53490	1009.8	79.88	796.2	722003	51.425	ng/ml
Topiramate	4.935	3497	287.9	47.16	1049.8	5831	51.391	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 5 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 5 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-E5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:40:13 PM		
Sample Info.			

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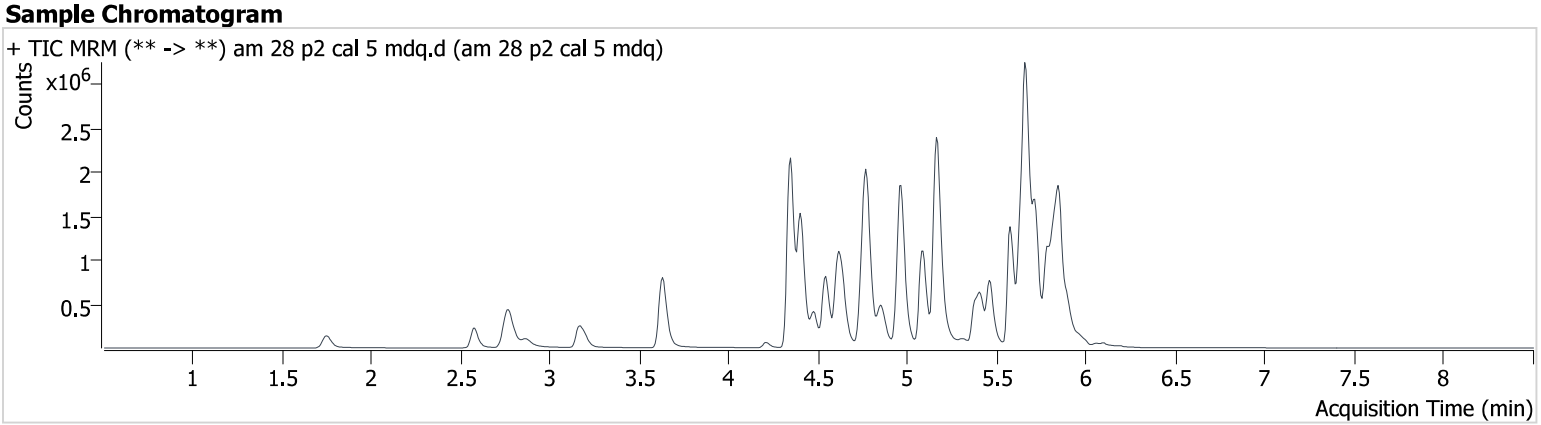
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	1028548	999540.9	90.93	7642.3	512088	94.396	ng/ml
methocarbamol	4.373	86320	4309.9	80.03	827.4	609337	98.724	ng/ml
Topiramate	4.935	6811	533.0	43.96	95.7	5423	107.186	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 6 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 6 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-F5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:49:27 PM		
Sample Info.			

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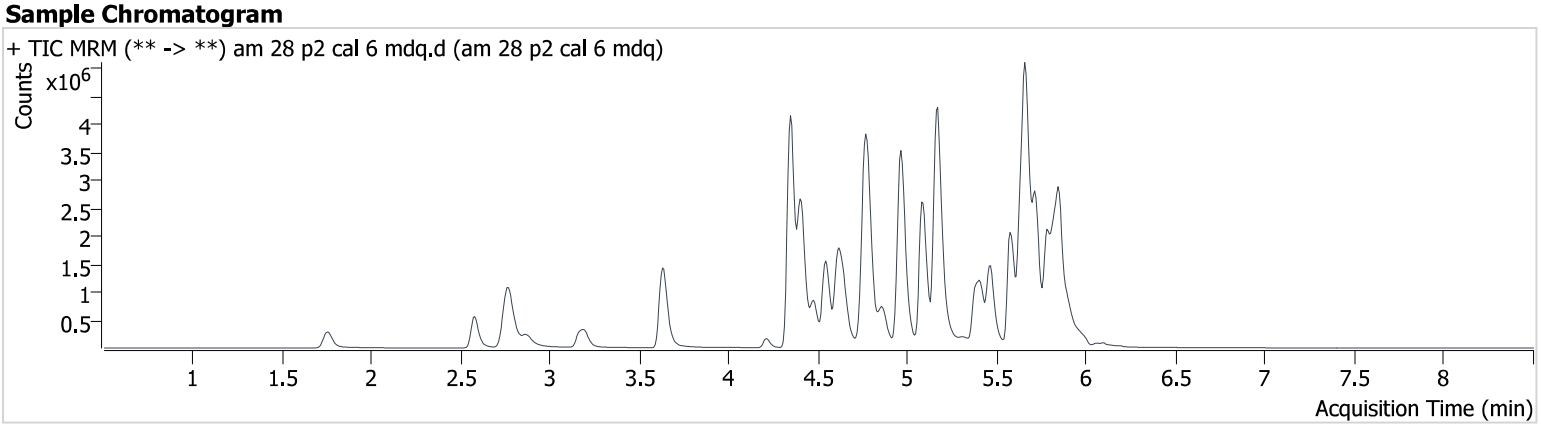
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	1979033	93500.1	83.74	33719.9	342175	271.288	ng/ml
methocarbamol	4.373	160047	2103.2	83.10	31270.2	453067	246.820	ng/ml
Topiramate	4.935	14730	6381.6	45.77	217.5	5306	236.466	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 7 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 7 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-G5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 5:58:40 PM		
Sample Info.			

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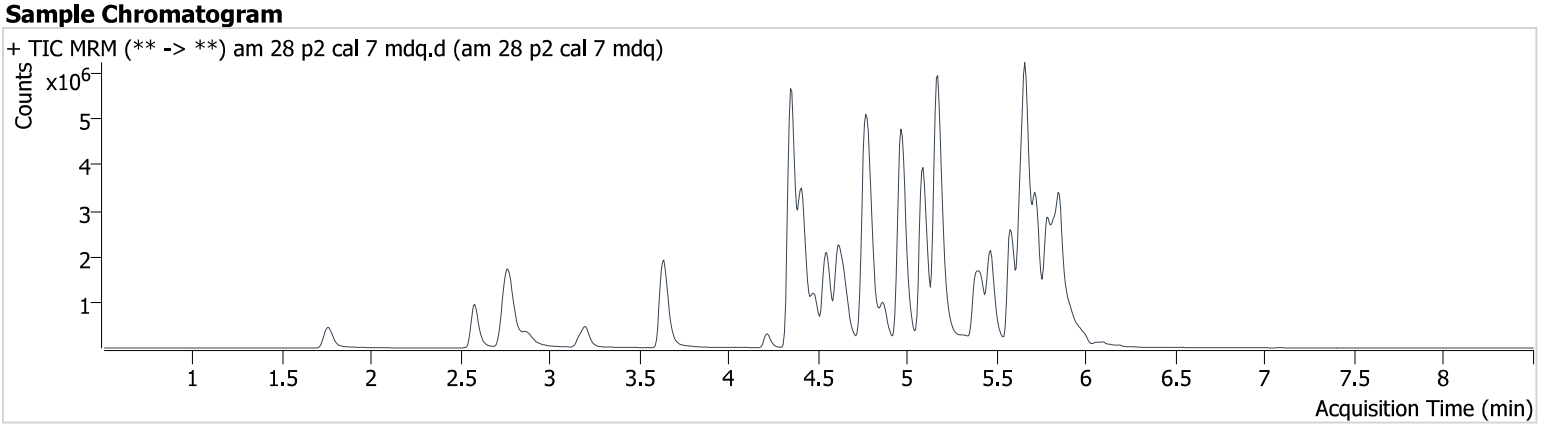
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	2325643	4411.7	90.03	7917.6	211114	516.461	ng/ml
methocarbamol	4.380	208233	23932.6	79.25	15457.4	287404	506.687	ng/ml
Topiramate	4.935	24790	641.4	44.19	455.1	4806	439.029	ng/ml

AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2024\am 27-28\111924\QuantResults\am 28 p2.batch samples.bin.batch.bin
Calibration Last Update 11/20/2024 4:33:20 PM

Instrument	69679	**Data File**	am 28 p2 cal 8 mdq.d
Type	Cal	**Sample**	am 28 p2 cal 8 mdq
Acq. Method	mdqp2 2023.m	**Operator**	Anne Nord
Sample Position	P2-H5	**Comment**	
Injection Volume	5		
Acq. Date-Time	11/19/2024 6:07:54 PM		
Sample Info.			

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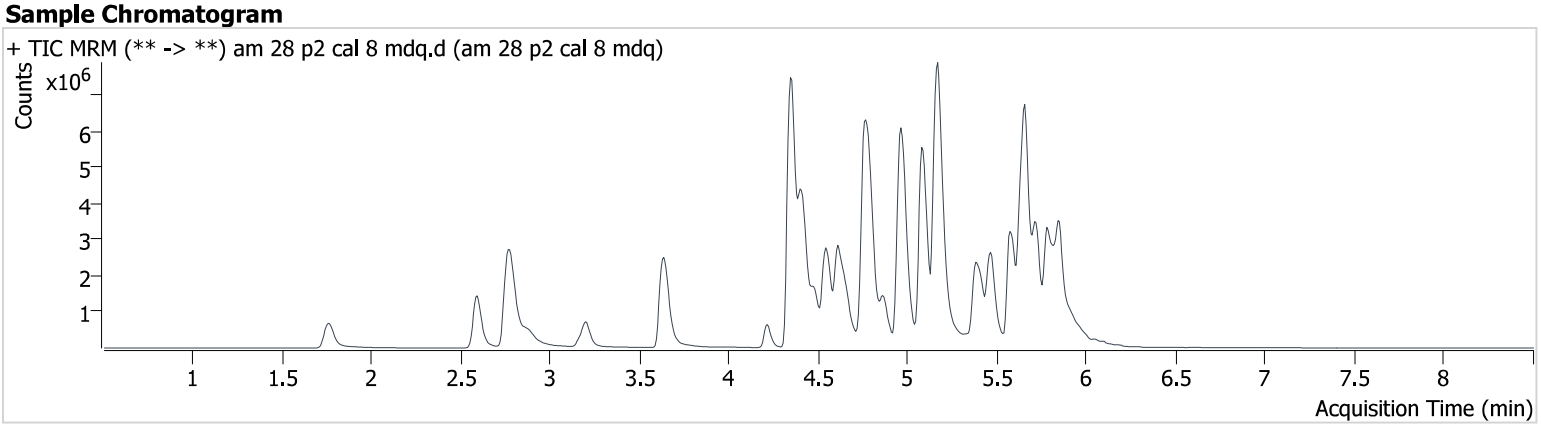
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
Clomipramine	5.849	2268731	12901.8	89.50	28246.1	97719	1088.153	ng/ml
methocarbamol	4.380	242112	60665.9	83.44	184538.9	161914	1046.174	ng/ml
Topiramate	4.935	37782	614.9	43.38	448.9	4960	648.083	ng/ml